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The effect of temperature change on metabolism: separating biological and chemical reactions

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Abstract. Life-detection experiments carried out in extraterrestrial locations provided inconclusive results whether processes observed were biological or chemical. In this study, the typical effect of temperature on metabolic rates is described and a life-detection method that is easy to perform is proposed. The method comprises observing changes in microbial metabolic rates after temperature shift. The method was demonstrated by experiments on aquatic microorganisms in the Gulf of Gdansk (Baltic Sea). First experiment, in which temperature was shifted within the temperature range encountered at the sampling site, demonstrated a typical Q_{10} coefficient (2.84). The experiment in which temperature was shifted beyond the environmental temperature range provided an unexpectedly low Q_{10} coefficient (1.44), which indicated that excessive temperature exerted an inhibitory effect on metabolism. This response is not expected for chemical reactions, but it is typical for biological processes. In summary, a pair of properly-tailored experiments permitted separating biological and chemical reactions.

Key words: temperature shift experiments; surface water; bacteria; Gulf of Gdańsk; oxygen consumption; life-detection.

1. Introduction

The most promising extraterrestrial sites that could host extant microbial life are subsurface oceans on jovian satellite Europa or saturnian satellite Enceladus and also regolith on Mars (Schulze-Makuch et al., 2015; Cockell et al., 2016). The only life-detection experiments were performed on Mars by landers of the Viking Mission in 1976. Unfortunately, since 1970s no subsequent metabolic experiments have been conducted in any extraterrestrial location. The Viking life-detection experiments were inconclusive and demonstrated difficulty in confirming or excluding the occurrence of extant life (Schuerger & Clark, 2008; Schulze-Makuch et al., 2015). Consequently, the question about life on Mars remains unanswered (Bianciardi et al., 2012; Levin & Straat, 2016; Guaita, 2017). It is worth mentioning that during all three biological experiments (the Labeled Release, the Pyrolytic Release, and the Gas Exchange or the Carbon Assimilation), the temperature was considerably elevated (to 8-26°C, depending on experiment; Horowitz et al., 1977; Klein, 1977; Levin & Straat, 1977; Oyama & Berdahl, 1977; Levin & Straat, 2016) as compared to that outside both Viking landers (-90°C to -5°C; Kieffer, 1976). This difference resulted from the heat sources inside the Viking landers (Horowitz et al., 1977) and could have influenced the results (Klein, 1977). However, this has not yet been discussed thoroughly.

In the present study, the typical impact of temperature on metabolic rate was described and demonstrated in experiments. How to use incubations performed at different temperatures to discriminate between biological and chemical processes is also proposed. According to the Van't Hoff rule, increases of temperature by 10°C cause increases of chemical process rates by a factor (Q_{10}) of between two and three. This rate of increase is also true of biological processes, however, only within a temperature range tolerated by the organism in question (reviewed by Fenchel,

2005). The Q_{10} of biological processes decreases close to the maximum tolerable temperature (reviewed by Fenchel, 2005). In summary, the response of biological reactions to temperature depends on the temperature range. Q_{10} factors estimated for whole communities of organisms in aquatic environments typically fall within a range of 2-3 (reviewed by del Giorgio & Williams, 2005), and the corresponding factor for soil-dwelling organisms ranges from about 1.5 to about 4, but it is typically around 2 (Rastogi et al., 2002; Barba et al., 2018). Thus, response of metabolic processes to temperature is universal for different communities of organisms. In this study, two experiments were performed to illustrate the response of respiration rates to temperature shifts – within and beyond the range of temperatures encountered in the environment. Coastal water with aquatic organisms was used as the material in order to keep the experiments simple in both design and execution and to easily define the temperature ranges to which the organisms are accustomed. At the sampling site (Baltic Sea, northern temperate zone) the water temperature ranges from about 0°C in winter to slightly above 20°C in summer (Rychert et al., 2015). Throughout the year, microbial community composition changes according to changing temperature, and microorganisms are adapted to typical temperatures (Sieburth, 1967). The experiments were conducted twice – in April when the temperature was elevated from environmental value of 5.5°C to 10.5°C, and in August when the temperature shifted from the environmental value of 22°C to 27°C. In the latter experiment, the temperature was raised above the range encountered in the environment. It was hypothesized that the Q_{10} coefficient calculated for the respiration rate in the first experiment (5.5→10.5°C, upshift within the environmental range of temperature) would be typical and the Q_{10} estimated in the second experiment (22→27°C, upshift beyond the environmental range) would be below typical values. Respiration (oxygen consumption) was chosen as the general index of metabolism. In aquatic environments, the main oxygen consumers are heterotrophic bacteria (del Giorgio & Cole, 1998); thus, their abundance was also estimated. To detect possible artefacts during incubation, the abundance of immediate bacterial grazers, i.e. protozoa, was also assessed.

2. Materials and Methods

Subsurface water for the experiments was collected in April and August 2002, from the end of a 450 m pier located in the Gulf of Gdańsk (54°27'N, 18°35'E, Baltic Sea). The water was filtered to exclude larger organisms (nylon screen, 25 µm pore size), mixed gently, and siphoned through gas-tight tubing into 1 L bottles (April – 6 bottles, August – 10 bottles) (Fig. 1). The bottles were incubated in the dark and with rotation (3 rpm). Darkness was necessary to exclude photosynthesis that produces oxygen and obstructs oxygen consumption measurements.

During the first 24 h, incubation was conducted at the environmental temperature (in April 5.5°C, in August 22°C). The bottles were taken sequentially for analyses after 0 h (2 bottles, both in April and August), 8 h (1 bottle, in August only), 16 h (1 bottle, August) and 24 h (2 bottles, April and August) (Fig. 1). After 24 h, the temperature was increased by 5°C (in April 10.5°C, in August 27°C). The remaining bottles were taken for analyses after 32 h (1 bottle, August), 40 h (1 bottle, August) and 48 h (2 bottles, April and August) (Fig. 1). Just before the second day of incubation, additional water was collected at the study site and prepared for incubation. This water (April – 4 bottles, August – 6 bottles) was incubated along with the water collected previously and only at an elevated temperature. The initial 2 bottles were taken for analyses after 24 h of the experiment (April and August), further bottles were taken after 32 h (1 bottle, August), 40 h (1 bottle, August) and 48 h (the two remaining bottles, April and August) (Fig. 1). In August the higher

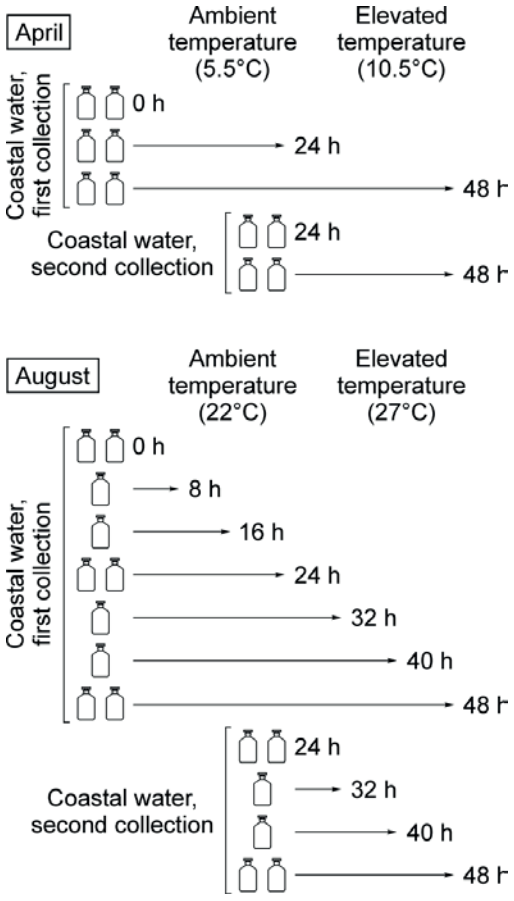

Figure 1. The scheme of temperature upshift experiments conducted using coastal water taken from the Gulf of Gdańsk (Baltic Sea). Water was siphoned into 1 L bottles, which were taken sequentially for the analyses of oxygen concentration.

sampling frequency confirmed stable respiration rates over 24 h of incubation. The newly collected water was incubated to detect possible resource limitation of bacterial growth during the prolonged 48 h incubation.

Water from the sampled bottles was siphoned into Winkler flasks (3 flasks per bottle) to measure oxygen concentrations. Measurements were performed with the standard Winkler's method. Titrations were conducted with an automatic piston titrator with potentiometric endpoint detection (Metrohm Titrino 702SM). Respiration rates were estimated as decreases in oxygen concentrations over time. Additionally, the abundance of bacteria (and possibly archaea) were estimated under an epifluorescence microscope after staining with acridine orange (Hobbie et al., 1977). Small flagellates (1-8 μm) were also counted under the epifluorescence microscope after fixation with glutaraldehyde and staining with primulin (Caron, 1983). In a few samples, ciliate abundance was studied under an inverted microscope using the Utermöhl (1931) method. Statistical calculations were performed with PAST v. 3.16 (Hammer et al., 2001).

3. Results

During both experiments, the water collected from the environment was well oxygenated (12.6 and 12.9 $mgO_2\ dm^{-3}$ in April, 8.5 and 8.6 $mgO_2\ dm^{-3}$ in August, Fig. 2), which permitted reliable respiration rate measurements. In April, after elevating the temperature from 5.5°C (environmental temperature) to 10.5°C, respiration increased from 0.54 $mgO_2\ dm^{-3}\ d^{-1}$ to 0.91 $mgO_2\ dm^{-3}\ d^{-1}$ (169% of the previous value, Fig. 2). The latter was only slightly lower than the value for the newly-collected water from the sampling site which was incubated at an elevated temperature – 1.05 $mgO_2\ dm^{-3}\ d^{-1}$. This indicated that a prolonged, two-day incubation period did not result in the significant resource limitation of bacterial metabolism. In August, after elevating the temperature from 22°C (environmental temperature) to 27°C, the increase in respiration was lower from 0.75 $mgO_2\ dm^{-3}\ d^{-1}$ to 0.90 $mgO_2\ dm^{-3}\ d^{-1}$ (120% of the previous value). The corresponding value for the newly-collected water was quite similar at 0.98 $mgO_2\ dm^{-3}\ d^{-1}$, which demonstrated that no resource limitation was observed during the two-day incubation period. The oxygen removal trend revealed by the high sampling frequency in this experiment demonstrated that respiration rates were stable during each day of incubation (Fig. 2). It excluded the occurrence of respiration rate resource limitation and indicated that respiration rates responded directly to temperature. Differences between respiration rates at environmental and elevated temperatures were statistically significant (Fig. 2).

The Q_{10} coefficient for the temperature difference of 10°C was calculated using an appropriate equation (Montagnes et al., 2003) for water incubated sequentially at environmental and later at elevated temperatures:

$$Q_{10} = (R2/R1)^{[10/(T2 - T1)]},$$

where R1 and R2 were respirations rates at environmental (T1) and experimentally elevated (T2) temperatures. In April, Q_{10} was 2.84 and in August it was 1.44. The value calculated for April was high and typical at between two and three (Table 1). The value obtained for the experiment performed in August, in which temperature was raised above the naturally occurring range at the sampling site, was below two. These experiment results were accurately hypothesized in the Introduction.

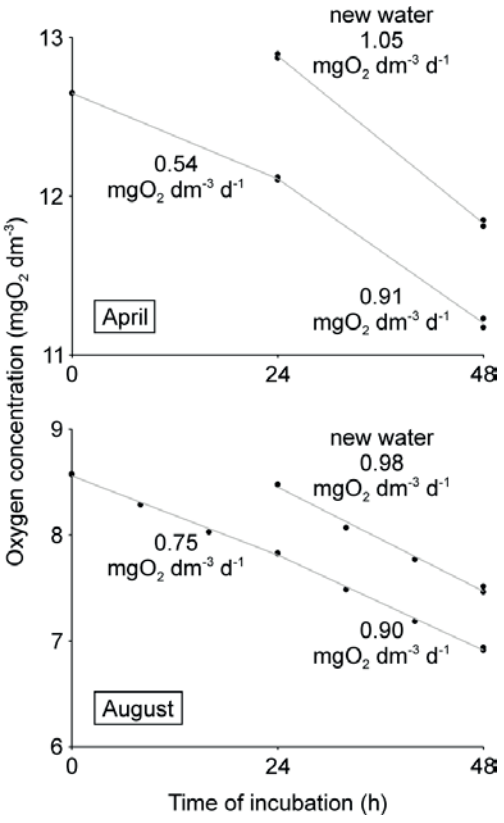

Figure 2. Dynamics of oxygen consumption during two temperature shift experiments (April, August) performed with coastal water collected from the Baltic Sea. During the first half of incubation (0-24 h), water was incubated at environmental temperatures. Later (24-48 h) the temperature was elevated by 5°C. Between 24 and 48 h, additional newly-collected water was incubated to detect possible resource limitation of microbial metabolism. Respiration rates were estimated based on parallel treatments, and mean respiration rates are indicated next to the curves. Differences between respiration rates at environmental and elevated temperatures were statistically significant according to Welch's ANOVA for unequal variances in April $p = 0.009$ ($F = 239$, df = 1.69) and August $p = 0.037$ ($F = 75.1$, df = 1.41).

Table 1. Q_{10} values calculated for respiration rates measured for microbial community in surface water collected from the Gulf of Gdańsk (Baltic Sea). Q_{10} value for temperature shift within the environmental range was typical (between 2 and 3) and Q_{10} value for temperature raised above the environmental range was lower than expected for chemical reactions. Thus, a pair of such experiments can prove the biological character of the process studied.

Experiment	Temperature shift	Q_{10} value
April	5.5→10.5°C (within the environmental range)	2.84 (typical)
August	22→27°C (beyond the environmental range)	1.44 (low)

4. Discussion

The respiration rates observed at environmental temperatures (0.54 mgO_2 dm^{-3} d^{-1} in April and 0.75 mgO_2 dm^{-3} d^{-1} in August) were within the broad range of values encountered in aquatic environments (del Giorgio & Williams, 2005) and similar to respiration rates reported previously from the coastal zone of the Gulf of Gdańsk (from near zero values to 1.28 mgO_2 dm^{-3} d^{-1}; York et al., 2001). The abundance of bacteria (with some archaea possibly among them) was estimated to characterize the microbial community. Their abundance was about 2×10^6 ml^{-1} in April and about 16×10^6 ml^{-1} in August. These values were similar to those previously reported from the coastal zone of the Gulf of Gdańsk (Witek et al., 1997; Rychert et al., 2015).

Prior to the experiments, water was screened through 25 µm nets to exclude larger organisms and detritus particles that could have been unevenly distributed among the bottles. However, truncating the trophic web can trigger the trophic cascade effect (Ripple et al., 2016), i.e. a phenomenon in which the removal of predators releases its immediate prey from grazing pressure allowing these organisms to multiply and intensify grazing on smaller organisms. The trophic cascade effect can only occur if a system is controlled by predation and not by resource availability. Another study (Rychert et al., 2015) indicated that this was not the case at the sampling site. Theoretically, trophic cascade can occur in the microbial food web of a 25-µm-filtrate: bacteria – flagellates – ciliates (Sherr & Sherr, 2002). To verify this possibility, changes in the abundance of bacteria (both in April and August), flagellates (both in April and August) and ciliates (in April) were assessed. In April, bacterial abundance increased during incubation, but the abundances of flagellates and ciliates were generally stable (data not shown). In August no systematic or significant changes in bacterial abundance were observed, and the abundance of flagellates was stable or slightly decreased, depending on incubation. In conclusion, no trophic cascade effect was observed during the experiments. The increase of bacterial abundance during the experiment in April demonstrated the "bottle effect" (Ferguson et al., 1984), which refers to the changes within a microbial community and the growth of specific bacteria that occurs in water confined to bottles. The "bottle effect" occurs in any experiment performed in confinement.

Temperature shift experiments are known in microbial ecology (e.g. Herbert & Bell, 1977) and are backed up by well-established ecological knowledge. In the present study, it was demonstrated that a pair of such experiments can prove the biological character of the process studied. Such studies can be performed with different metabolic processes including anaerobic ones, e.g. decomposition of organic matter or some kind of chemosynthesis. In the future, temperature shift experiments could be carried out on extraterrestrial worlds like Europa (Jupiter's satellite) and Enceladus (Saturn's satellite), which could host living organisms in their subsurface oceans (Cockell et al., 2016).

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